**2059-Pos Board B45**
**Transporter BetP - stimulus Sensing and Oligomeric Structure**
**Reinhard Kraemer**, Markus Becker, Stanislaw Maksimow, Christine Ziegler.
The Na+ coupled betaine uptake system BetP of Corynebacterium glutamicum belongs to the BCCT family of transporters and comprises both a catalytic function (betaine/Na+ cotransport) and a sensory/regulatory function (responding to osmotic stress). Its 2D (electron crystallography) and 3D structure (X-ray crystallography) has been solved. Within a homooligomeric trimer, each BetP protomer harbours both an N- and a C-terminal domain involved in stimulus sensing and intramolecular signal transduction. Factors known so far contributing to the sensory and regulatory function of BetP are (i) the two terminal domains, (ii) K+ ions as an osmostress related stimulus, and (iii) interaction with the surrounding membrane.
The primary stimulus of BetP, the rise in the cytoplasmic K+ concentration, has been elucidated using a proteoliposomal system, whereas the second stimulus, attributed to changes in the physical state of the surrounding membrane was investigated in intact cells. We have now succeeded to discriminate the two stimuli in the two different experimental systems and to quantify their impact on activation of BetP.
Intramolecular signal transduction of the two different stimuli involves contributions from individual domains of BetP and is essentially based on its oligomeric (trimeric) structure. This was elucidated in mechanistic terms using a newly developed heterooligomeric construct of BetP composed of three different protomers.
On the basis of these studies we suggest a functional model of intersubunit crosstalk between the three individual BetP monomers as well as the terminal domains of BetP during its catalytic and its sensory function.

**2060-Pos Board B46**
**Crystal Structures of the CusA Efflux Pump Suggest Methionine Mediated Metal Transport**
**Edward Yu**.
Gram-negative bacteria, such as Escherichia coli, frequently utilize tripartite efflux complexes in the resistance-nodulation-cell division (RND) family to expel diverse toxic compounds from the cell. The efflux system CusCBA is responsible for extruding biocidal Cu(I) and Ag(I) ions. No prior structural information was available for the heavy-metal efflux (HME) subfamily of the RND efflux pumps. Here we describe the crystal structures of the inner membrane transporter CusA in the absence and presence of bound Cu(I) or Ag(I). These CusA structures provide important new structural information about the HME sub-family of RND efflux pumps. The structures suggest that the metal binding sites, formed by a three-methionine cluster, are located within the cleft region of the periplasmic domain. Intriguingly, this cleft is closed in the apo-CusA form but open in the CusA-Cu(I) and CusA-Ag(I) structures, which directly suggests a plausible pathway for ion export. Binding of Cu(I) and Ag(I) triggers significant conformational changes in both the periplasmic and transmembrane domains. The crystal structure indicates that CusA has, in addition to the three-methionine metal binding site, four methionine pairs - three located in the transmembrane region and one in the periplasmic domain. Genetic analysis and transport assays suggest that CusA is capable of actively picking up metal ions from the cytosol, utilizing these methionine pairs/clusters to bind and export metal ions. These structures suggest a stepwise shuttle mechanism for transport between these sites.

**2061-Pos Board B47**
**Hydrophobic Patch is Indispensable for Passenger Domain Translocation of Autotransporter**
**Yujia Zhai**.
Whooping cough (pertussis) is a highly contagious, acute respiratory illness of humans caused by the gram-negative bacterial pathogen Bordetella pertussis. Autotransporter (AT) BrkA is an important B. pertussis virulence factor that confers serum resistance and mediates adherence. Here we present the crystal structure of B. pertussis BrkA β domain at 3Å resolution. It shows that a hairpin-like structural motif from an adjacent molecule is inserted into the central pore of the β barrel. An undiscovered hydrophobic cavity formed by the hydrophobic patches on the loop L4, β-strands S5 and S6 adopts a ubiquitous structural characteristic of all AT β domains. A mutagenesis study proved that the hydrophobic cavity is indispensable for BrkA passenger domain translocation. This structure helps in understanding the molecular mechanism of AT secretion and provides a potential target for anti-pertussis drug design.

**2062-Pos Board B48**
**High-Resolution Structure of the *Vibrio Cholerae* Cytolysin Heptamer**
**Swastik De**, Rich Olson.
*Vibrio cholerae* cytolysin (VCC) is a β-pore forming toxin (β-PFT) that helps the devastating cholera pathogen survive within and colonize the human digestive tract. After being secreted as a water-soluble monomer (~80 kDa), VCC is cleaved by proteases to form the mature toxin (~65 kDa). Like many PFTs, mature VCC protomers self-assemble on cell membranes forming a transmembrane channel or pore, which causes damage or death by osmotic lysis. This may play a role in protecting the pathogen from immune cells while causing damage to the intestinal epithelium. Lack of high-resolution structures of assembled pore-forming toxins has limited our understanding of toxin assembly and pore formation.
We solved the 2.9 Å structure of detergent-solubilized VCC by X-ray diffraction from toxin assembled on cholesterol/asolectin liposomes. This structure, along with our previous structure of the VCC monomer, reveals details of domain rearrangements that occur during pore formation. Comparison of the monomer and the heptamer structures provides insight into why this toxin requires specific lipids and cholesterol in the cell membrane for assembly and pore formation. The charge distribution within the channel helps to explain the anionic rectification behavior observed in planar lipid recordings. This is the first example of a β-PFT for which soluble pro-toxin and assembled pore structures have been solved to high resolution and may provide a basis for therapeutic strategies that target or utilize the VCC toxin.

**2063-Pos Board B49**
**Novel Amphiphilic Molecules Mediate Membrane Protein Crystal Contacts**
**Qinghai Zhang**.
There have been quite a few approaches developed over the last two decades towards the creation of structurally novel amphiphiles. Some of these reagents have found broad applications in membrane protein biochemistry, being used for the solubilization and stabilization of membrane proteins for functional studies. However, success has been very limited in using these novel amphiphiles to crystallize IMPs. The lack of success exemplifies the significant degree of challenge and makes it clear that other types of amphiphiles are needed. We have contributed hundreds of new detergents through our previous efforts in this area. These detergents have been tested for various purposes (e.g. solubilization, stabilization, NMR, crystallization), and several were found to perform as well or better than the most popular commercial detergents. In particular, the use of our newly designed cholesterol-like facial amphiphiles has facilitated the 3D crystallization and structural determination of several membrane proteins. Interestingly, we have found evidence that facial amphiphiles mediated protein crystal contact. These results open up the possibility for future investigations of using intelligently designed stabilization reagents to mediate membrane protein surface interactions so as to increase crystallization propensity and improve crystal diffraction.

**2064-Pos Board B50**
**On the Interaction of Large Amounts of C12E8 on Na,K-ATPase Alpha Subunits: A Small Angle X-Ray Scattering Study**
**Leandro R.S. Barbosa**, Carolina Rigos, Juliana Y. Sakamoto, Rosangela Itri, Pietro Ciancaglini.
In the current work, we studied the effect of the non-ionic detergent dodecyloctaethyleneglycol, C12E8, on the structure and oligomeric form of Na,K-ATPase membrane enzyme (sodium-potassium pump) in aqueous suspension, by means of small angle X-ray scattering (SAXS). Solubilized samples composed of 2 mg/ml of Na,K-ATPase, extracted from rabbit kidney medulla, in the presence of small C12E8 amount (0.005 mg/ml) and in larger concentrations ranging from 2.7 mg/ml to 27 mg/ml did not present catalytic activity. Under this condition, an oligomerization of the alpha sub-units is expected. SAXS data were analyzed by means of a global fitting procedure supposing that the scattering is due to two independent contributions: one coming from the enzyme and the other one from C12E8 micelles. In the small detergent content (0.005 mg/ml), the SAXS results evidenced that Na,K ATPase is associated into aggregates larger than alpha2 form. When 2.7 mg/ml of C12E8 is added, the data analysis revealed the presence of alpha4 aggregates in the solution and some free-micelles. Increasing detergent amount up to 27 mg/ml does not disturb the alpha4 aggregate, just more micelles of same size and shape are proportionally formed in solution. We believe that our results shed light to better understanding on how non-ionic detergents induce sub-units dissociation and reassembling to minimize the exposure of hydrophobic residues to the aqueous solvent.
Acknowledgments: the authors thank FAPESP CAPES and CNPq for financial support.